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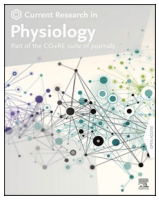

Yeast supplementation potentiates fluoxetine's anti-depressant effect in mice via modulation of oxido-inflammatory, CREB, and MAPK signaling pathways

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ABSTRACT

Introduction: The therapeutic potential of yeast in the management of depression is unknown. Thus, we evaluated the modulatory effect of nutritional yeast supplementation on antidepressant activity of fluoxetine in mice models of depressive-like behaviors (DLB).

Methods: A total of 112 mice were divided into 16 groups (n = 7 each) for a 3-stage study. Stage I (non-DLB study) had groups Ia (10 mL/kg vehicle), Ib (20 mg/kg fluoxetine), Ic – If (2% yeast diet for all, but Id - If additionally received 5 mg/kg, 10 mg/kg, and 20 mg/kg fluoxetine respectively). Stage II (lipopolysaccharide [LPS] model of DLB) had groups IIa - IIb (10 mL/kg vehicle), IIc (20 mg/kg fluoxetine), IId (yeast) and IIe (yeast + 20 mg/kg fluoxetine). After these treatments for 24 days, animals in IIb - IIe received 0.83 mg/kg of LPS on the 25th day. Except for group IIIa (10 mL/kg vehicle), animals in other groups of stage III (unpredictable chronic mild stress [UCMS] model) were exposed to UCMS for 24 days along with 10 mL/kg vehicle (IIIb), 20 mg/kg fluoxetine (IIIc), yeast (IIId), or yeast + fluoxetine (IIIe).

Results: Yeast and fluoxetine attenuated LPS- and UCMS-induced immobility, derangement of oxido-inflammatory (TNF-α, IL-6, NO, MDA, SOD, GSH, CAT, and AChE) and CREB/MAPK pathways. While fluoxetine had more potent effect than yeast when used separately, pre-treatment of mice with their combination had more pronounced effect than either of them.

Conclusion: Yeast supplementation improves the antidepressant activity of fluoxetine in mice by modulating oxido-inflammatory, CREB, and MAPK pathways.

1. Introduction

Globally, the World Health Organization reported that more than 350 million people of all ages suffer from depression, making major depressive disorder (MDD) a serious mental disorder of public health concern (Woodward et al., 2013; Zalar et al., 2018; Alemayehu et al., 2019). Depression represents the largest share (10.3%) of the total disease burden, causing 76.4 million years lived with disability (YLD) worldwide (Zalar et al., 2018). Many researchers have hypothesized different theories for the pathogenesis of depression, including heritability, neurotransmitter systems, brain-derived neurotrophic factor (BDNF), and the overactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Peng et al., 2015).

Inflammation in the central nervous system (CNS) has been well-established to lead to the development of depression and the neuro-inflammation hypothesis of depression has been widely documented by scientists. For instance, elevation of inflammatory biomarkers is often noticed in depressed patients (Mazza et al., 2021), while persistent inflammatory activation can elicit depression in susceptible persons (Slavich and Irwin, 2014; Omeiza et al., 2021). Experimentally,

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injection of bacterial lipopolysaccharides (LPS) in humans leads to increased plasma pro-inflammatory cytokines and dose-dependent decreased mood (Grigoleit et al., 2011). That anti-depressant drugs like fluoxetine (selective serotonin reuptake inhibitor), clomipramine (tricyclic antidepressant), and tranylcypromine (monoamine oxidase inhibitor) can prevent LPS-induced microglial changes and production of pro-inflammatory markers like interleukin 1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) suggests that suppression of neuroinflammation is one of their key anti-depressant mechanisms (Mao et al., 2019; Mariani et al., 2022). Thus, animal models of neuroinflammation-associated depressive-like behaviors (DLB) are most commonly created by administration of LPS.

Irresolvable psychological stress significantly contributes to the development of depression. Various studies have used several animal models of stress-induced DLB, including social defeat stress, early life stress, learned helplessness, among others, all of which have been shown to have inherent disadvantages that lessen their translational potentials (Baune et al., 2012). The unpredictable chronic mild stress (UCMS) protocol is a popular model that has been adjudged to be one of the translationally-relevant models of studying the pathophysiology of DLB in rodents (O'Leary and Cryan, 2013). It uses mild stressors rather than aggressive or early-life stimuli, and elicits DLB that are comparable to clinical symptoms of depression, including anhedonia (decreased responsiveness to rewards), helplessness and despair, deterioration of the coat state, and altered sexual activity (Mineur et al., 2006). This unpredictable and uncontrollable stressor has the ability to cause impairment of the micro-vascular functions of the skeletal muscle and the cerebrum (Jayatissa et al., 2010; Branyan et al., 2018). It has been used to demonstrate all symptoms of depression, which were reversed by chronic treatment with various anti-depressants (Mutlu et al., 2012; Isingrini et al., 2012), suggesting similarity of its depressive-like effect with humans and its translational efficacy.

Despite the availability of many antidepressants, an inadequate response still occurs in approximately 30% patients (Peng et al., 2015) while more than 85% of them with a first episode relapses after some years. Moreover, most of the patients have suicidal thoughts, with 15–20% dying from suicide (Crane et al., 2014). Reports have also shown that about 20%–30% of depressed patients do not respond to available pharmacological treatments, as they are based on the monoamine hypothesis. With growing concerns about the therapeutic failure of standard anti-depressant medications, it is pertinent to look into other aspects such as nutrition and the gut-brain axis for possible interventions to depression.

During the past decade, there has been an increase in understanding of how the gut microbiota affects various aspects of brain development and function, as well as behavior. For example, studies on germ-free mice revealed that gut bacteria influence development of stress response, appropriate maturation and function of microglia, affect anxiety, social and DLB, along with alterations in gene expression and neurochemistry of different brain regions (Erny et al., 2015; De Palma et al., 2015). Factors such as psychological stress and diseases impairing one or more pathways of the gut-brain axis result in depression (Liang et al., 2018). Moreover, depressed patients often have gut-brain dysfunction, such as disturbances in appetite, metabolic and gastrointestinal functions, and gut microbiota (Lach et al., 2018). The importance of gut bacteria in the development of mood disorders was further confirmed by several recent studies showing that patients with depression had altered diversity and composition of gut microbiota, and these changes were causally related to DLB in rodent models (Tian et al., 2022). The gut microbiota hypothesis posits that depression is closely related with gut microbiota, and that the microbiota–gut–brain axis dysfunction is the main pathological basis of depression. Thus, microbiota regulation is a promising method for depression therapy and prevention. An increasing amount of research exploring the gut-brain axis in the last decades supports the hypothesis from different aspects (Liang et al., 2012; Rieder et al., 2017).

Diet is one of the most influential factors on the gut microbiota after weaning, and poor or unhealthy diets that contain excessive saturated fat, sugar, and food additives, including the Western diets, the refined-food diet, and industrially-processed food, significantly perturb microbiota and increase the incidence of depression (Yatsunenko et al., 2012; Slyepchenko et al., 2017). Nutritional yeast, also known as "hippie dust, " is a single-cell eukaryotic probiotics (fungus) with an incredible nutritional value. It's made from *Saccharomyces cerevisiae*, a type of yeast used in brewing and baking (Johansen et al., 2019). Yeast, besides its importance in food fermentation, has also shown numerous beneficial effects on human health. In addition to its immunomodulatory effects, Yeast's probiotic effects, including the prevention and treatment of intestinal diseases, are the most well-known health effects (Mufandaedza et al., 2006; Motlhanka et al., 2018). It delivers a blast of high-quality protein, fiber, B vitamins, and minerals (Johansen et al., 2019) and also transforms certain molecules into biologically active compounds (Motlhanka et al., 2018), e.g. amino acid tryptophan, which is the precursor of at least three biologically-active compounds: melatonin, serotonin, and tryptophol (Takada et al., 2018).

Despite the nutritional benefits of yeast and its probiotic effect in the gut, it is not clearly understood whether it could influence the gut-brain axis to prevent or ameliorate depression. It is not also known if it could potentiate anti-depressant drugs to produce a better therapeutic effect. The present study sought to investigate (i) whether yeast, when used alone or in combination with fluoxetine, can prevent the development of DLB following LPS or UCMS challenge, and (ii) whether modulation of oxido-inflammatory, MAPK, CREB, and MAPK pathways are involved in the possible anti-depressant effect of yeast, either when used alone or when combined with fluoxetine. We hypothesized that yeast, when used alone or in combination with fluoxetine, would prevent the induction of DLB by LPS and UCMS models via modulation of these pathways.

2. Materials and methods

2.1. Drugs and reagents

The following agents were obtained from various sources: fluoxetine capsules (Fidson Healthcare PLC, Lagos, Nigeria), nutritional yeast (Kunimed Pharmachem Limited, Lagos, Nigeria), lipopolysaccharide (LPS; E. coli, serotype 055:B5) (Sigma-Aldrich, St. Louis, USA), ELISA kits for TNF-α and IL-6 (Bio Legend, USA), CREB and MAPK ELISA kits (Wuhan Fine Biotech Co., Ltd, China), and corticosterone ELISA kit (Oxford Biomedical Research, USA). Fluoxetine doses (5, 10, 20 mg/kg, p.o.) and LPS dose (0.83 mg/kg, i.p.) were chosen based on previous studies by Emslie et al. (2002), Kryst et al. (2022) and Painsipp et al. (2011). To avoid contamination, a 2% yeast-enriched meal was made every day during the course of the experiment.

Ethical statement

A total of 112 male Balb/c strain mice, each weighing approximately 25 g, were obtained from the Central Research Animal House at the University of Ibadan, Ibadan, Nigeria and used for this study. The mice were bred and housed in polystyrene cages measuring 42 cm in length, 30 cm in breadth, and 27 cm in height (n = 5 mice/cage). They were maintained under standardized conditions, including a temperature of 25 ± 1 °C, relative humidity of 60 ± 5%, and a 12-h light/dark cycle starting at 7:00 a.m. The animals were provided with standard pelletized rodent chow and potable water *ad libitum*. All protocols involving the use of experimental animals were properly adhered to, especially according to the criteria specified in the National Institutes of Health (NIH) (1986) "Guide for the Care and Use of Laboratory Animals". The study protocol received ethical approval with number UI-ACUREC/086–0921/13 from the University of Ibadan Animal Care and Use Research Ethics Committee. Every effort was made to uphold the principles of 3Rs in animal research, prioritizing the humane treatment of

the experimental animals.

2.2. Experimental protocols

The research work was carried out in three (3) stages: stage I was the non-DLB study while stages II (LPS model) and III (UCMS model) were the DLB studies (Scheme 1).

The non-DLB study was designed to establish the ordinary effect of yeast and/or fluoxetine on the neurobehavior of the animals. The studies on the LPS and UCMS models of DLB were designed to induce oxido-inflammatory and stress conditions respectively in the animals and investigate whether yeast and/or fluoxetine produce anti-depressant-like effect via modulation of the oxido-inflammatory pathways in these 2 models, as neuro-inflammation and stress have both been severally linked to DLB. Experiments in these 3 stages were conducted on animals after acclimatizing them for two weeks, weighing and random allocation to treatment groups in a blinded fashion.

The animals in the non-DLB study were allotted into six (6) oral treatment groups of seven (7) animals each, numbered Ia - If. Group Ia received 10 mL/kg of vehicle (normal saline) while group Ib received 20 mg/kg of fluoxetine. Groups Ic – If all received 2% yeast diet, but groups Id - If additionally received 5 mg/kg, 10 mg/kg, and 20 mg/kg fluoxetine (Emslie et al., 2002; Kryst et al., 2022) respectively. All the treatments were for 24 days, after which only neurobehavioral analysis were carried out as there was no sign of DLB in the animals.

The animals in the LPS model of DLB were allotted into five (5) groups of seven (7) animals each, numbered IIa – IIe. Groups IIa and IIb were given 10 mL/kg of vehicle, while group IIc received 20 mg/kg of fluoxetine. Both groups IId and IIe were fed a 2% yeast diet; however, group IIe was also administered an additional 20 mg/kg of fluoxetine. After these treatments for 24 days, animals in groups IIb – IIe were treated with 0.83 mg/kg of LPS on the 25th day. On the 26th day, neurobehavioral assessments were carried out, after which the animals were sacrificed to harvest brain samples for biochemical analysis.

The animals in the UCMS model of DLB were divided into five (5) groups, each consisting of seven (7) animals, numbered IIIa – IIIe. All groups except IIIa were exposed to UCMS throughout the 24-day experimental period, in addition to the specific treatments explained herein. Group IIIa and IIIb were given 10 mL/kg of vehicle, while group IIIc received 20 mg/kg fluoxetine. Groups IIId and IIIe both received 2% yeast diet, but group IIIe additionally received 20 gm/kg fluoxetine. On the 25th day, neurobehavioral assessments were carried out, after which the animals were sacrificed to harvest brain samples for biochemical analysis.

The animals were euthanized under ether anesthesia, and their brains were promptly removed, weighed, and homogenized in a 10% w/v sodium phosphate buffer (0.1 M, pH 7.4). Thereafter, the supernatants from the brain tissue homogenates were stored at −20 °C until biochemical analysis.

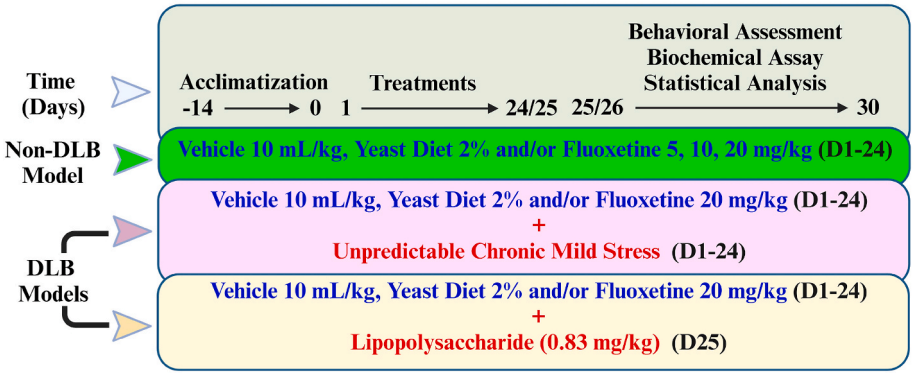
Apart from corticosterone, CREB, and MAPK that were only measured in animals under the UCMS model of DLB, the following parameters were measured in both DLB models: Immobility time, MDA, Nitrite, GSH, CAT, SOD, AChE, TNF-α, and IL-6.

2.3. Induction of DLB

LPS was utilized to induce neuroinflammation, a key factor in the development of DLB. This approach was based on the procedures detailed by Sulakhiya et al. (2015), which have been established as effective for eliciting DLB-like symptoms in experimental models. The UCMS method was used to induce chronic stress in mice, following the protocol outlined by Yalcin et al. (2005) with some modifications (Table 1). This approach aimed to maximize unpredictability by applying stressors in a seemingly random order and at various times post-drug administration. Each mouse was exposed to a minimum of two stressors daily. The UCMS protocol was implemented continuously for 24 days. Behavioral assessments were conducted 24 h after the final stressor to mitigate stress and treatment effects. Subsequently, blood samples were collected to measure serum corticosterone levels.

Table 1
Unpredictable chronic mild stress protocol.

Day	Daytime: Light Cycle (7 a.m.–7 pm) -stressors and duration	Overnight: Dark Cycle (7 p.m.–7 am) - stressors and duration
1	Forced swim (5 min)	Food deprivation (12 h)
2	Damp sawdust (120 min)	Hypoxia (15 min)
3	Change of cage mate (90 min)	Sawdust free cage (90 min)
4	45° cage tilting (120 min)	Water deprivation (12 h)
5	Overcrowding (30 min)	Background noise (30 min)
6	Starvation	Illumination overnight
7	45° cage tilting (120 min)	Water deprivation (12 h)
8	Forced swim (5 min)	Food deprivation (12 h)
9	Damp sawdust (120 min)	Hypoxia (15 min)
10	45° cage tilting (120 min)	Water deprivation (12 h)
11	Overcrowding (30 min)	Background noise (30 min)
12	Introduction of foreign object (big stone)	–
13	Forced swim (5 min)	Food deprivation (12 h)
14	Damp sawdust (120 min)	Hypoxia (15 min)
15	Change of cage mate (90 min)	Sawdust free cage (90 min)
16	45° cage tilting (120 min)	Water deprivation (12 h)
17	Overcrowding (30 min)	Background noise (30 min)
18	Introduction of foreign object (big stone)	–
19	Forced swim (5 min)	Food deprivation (12 h)
20	Damp sawdust (120 min)	Hypoxia (15 min)
21	Change of cage mate (90 min)	Sawdust free cage (90 min)
22	45° cage tilting (120 min)	Water deprivation (12 h)
23	Overcrowding (30 min)	Background noise (30 min)
24	Introduction of foreign object (big stone)	–



Scheme 1. Experimental protocol.

2.4. Behavioral assessment

The effects of nutritional yeast supplementation and/or the antidepressant fluoxetine on immobility time, an index of DLB, were assessed using the Forced Swim Test (FST) and Tail Suspension Test (TST). In the FST, based on the procedure described by Porsolt et al. (1978), mice were placed in a glass cylinder 20 cm tall and 10 cm in diameter, filled with water to a depth of 15 cm, leaving a 15 cm air space above the water level. The water temperature was maintained at room temperature, and the mice were observed for 15 min to measure immobility time.

For the Tail Suspension Test (TST), mice were suspended by their tails using adhesive tape on a horizontal bar, positioning their bodies approximately 15 cm above a surface to allow free hanging. The duration of immobility was recorded over a 6-min observation period. Immobility was defined as the absence of any limb or body movement, excluding movements due to breathing, following the standard protocol by Steru et al. (1985). The TST assesses behavioral despair, with increased immobility suggesting depressive-like behavior in rodents (Omeiza et al., 2021).

2.5. Biochemical assays

2.5.1. Determination of brain concentration of reduced glutathione (GSH)

The concentrations of GSH in brain tissues were determined using the method described by Moron et al. (1979). In brief, a 0.4 mL aliquot of each mouse's brain tissue homogenate was mixed with 0.4 mL of 20% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm at 4 °C for 20 min. The resulting supernatant (0.25 mL) was then mixed with 3 mL of 0.2 M sodium phosphate buffer (pH 8.0) and reacted with 2 mL of 0.6 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB). The absorbance at 412 nm was subsequently measured to quantify GSH concentration (expressed as μmol/g protein).

2.5.2. Determination of lipid peroxidation (Malondialdehyde [MDA])

The brain concentration of MDA, a prominent biomarker of lipid peroxidation and tissue injury, was determined using the method described by Okhawa et al. (1979). In brief, a 0.5 mL aliquot of brain homogenate was mixed with 0.5 mL of distilled water and 1.0 mL of 10% trichloroacetic acid (TCA), followed by centrifugation at 3000 rpm for 10 min. The resulting supernatant (0.9 mL) was then treated with 0.1 mL of 0.375% thiobarbituric acid (TBA) and incubated in a water bath at 80 °C for 40 min. After cooling to room temperature, the absorbance was measured at 532 nm to quantify MDA concentration (mol/g protein).

2.5.3. Determination of acetylcholinesterase (AChE) activity

The activity of brain AChE, a key indicator of cholinergic nervous system function, was assessed using Ellman's method (1961). In brief, a 0.4 mL aliquot of brain homogenate was incubated with 2.6 mL of 0.1 M phosphate buffer (pH 7.4), 0.1 mL of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), and 0.1 mL of acetylthiocholine. The reaction was monitored at 412 nm using an ELISA plate reader, with absorbance measurements taken every 2 min for 10 min. AChE activity was then calculated based on the rate of thiocholine formation (expressed as μmoles/min/g protein).

2.5.4. Determination of superoxide dismutase (SOD) activity

The SOD activity in mouse brains was evaluated using the method described by Misra and Fridovich (1972). In brief, a 0.1 mL aliquot of brain homogenate was incubated with 2.6 mL of 0.05 M carbonate buffer, 0.1 mL of distilled water, and 0.3 mL of 0.3 mM adrenaline. The mixture was rapidly inverted and mixed, and the absorbance at 480 nm was measured every 60 s for 3 min using an ELISA plate reader. SOD activity was quantified as units of adrenaline consumed per mg protein (units/mg protein), providing a measure of the enzyme's antioxidant activity.

2.5.5. Determination of catalase activity

Catalase (CAT) activity was determined using Sinha's method (1972), which is based on the enzyme-catalyzed breakdown of hydrogen peroxide (H_2O_2). Briefly, a 1 mL aliquot of brain homogenate was diluted 20-fold with distilled water (19 mL), and then 1 mL of this diluted solution was mixed with 5 mL of phosphate buffer (pH 7.0) and 4 mL of H_2O_2 (800 μmoles). The reaction mixture was gently swirled at room temperature, and then 1 mL of the mixture was transferred to a dichromate/acetic acid reagent (2 mL). The absorbance changes at 570 nm were measured at 60-s intervals using an ELISA plate reader to quantify catalase activity. The enzyme activity was expressed as the rate of H_2O_2 decomposition per unit protein.

2.5.6. Determination of nitrite content

Nitrite concentrations, a surrogate marker of nitric oxide production, was measured in mouse brain tissue using the Griess reagent method (Green et al., 1982). Briefly, a 100 μL aliquot of each mouse's brain tissue homogenate was incubated with an equal volume of Griess reagent, a 1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-1-naphthyl ethylenediamine dihydrochloride. The absorbance of the resulting mixture was then measured at 540 nm, and the nitrite content was determined by reference to a standard curve generated with sodium nitrite (0–100 μM).

2.5.7. Determination of mitogen-activated protein kinase (MAPK) levels

MAPK levels were evaluated using a MAPK ELISA Kit (Wuhan Fine Biotech Co., Ltd, China) based on sandwich enzyme-linked immunesorbent assay method. The kit used pre-coated anti-MAPK antibodies on 96-well plates and biotin-conjugated detection antibodies. Standards, test samples, and detection antibodies were added sequentially, followed by washes with wash buffer. HRP-Streptavidin was then added, and unbound conjugates were washed away. TMB substrates were used to visualize the HRP enzymatic reaction, producing a yellow color proportional to the captured MAPK amount. Absorbance was measured at 450 nm using a microplate reader, and MAPK concentrations were calculated.

2.5.8. Determination of cAMP response element binding protein (CREB) levels

CREB levels were measured using a CREB ELISA kit (Wuhan Fine Biotech Co., Ltd, China). The pre-coated microtiter plate was incubated with standards or samples, biotin-conjugated CREB antibody, and Horseradish Peroxidase (HRP)-conjugated Avidin. TMB (3,3′,5,5′ tetramethyl-benzidine) substrate was then added, and the enzyme-substrate reaction was terminated with sulfuric acid. The color change was measured spectrophotometrically at 450 nm ± 2 nm. The CREB concentrations in samples were determined by comparing the absorbance to a standard curve.

2.5.9. Determination of tumor necrosis factor α (TNF-α) and Interleukin-6 (IL-6) concentrations

TNF-α and IL-6 concentrations in brain tissue were measured using a Biolegend ELISA kits (Bio Legend ELISA MAX™ Deluxe kit, USA) according to the manufacturer's instructions. Reagents, standards, and samples were equilibrated to room temperature before use. The microplate, pre-coated with biotinylated mouse TNF-α and IL-6, was incubated with samples, standards, controls, and streptavidin-peroxidase conjugate for 2 h. Chromogen substrate was then added, followed by a 20-min incubation and Stop solution. Absorbance was measured at 450 nm using a microplate reader, and unknown sample concentrations (pg/mL) were determined using a log-log logistic curve-fit.

2.6. Statistical analysis

The data were expressed as means ± standard error of mean (S.E.M.). To compare mean values across groups, a one-way analysis of

variance (ANOVA) was conducted, followed by a Tukey post hoc test using GraphPad Prism Biostatistics software version 8. The significance level for all tests was set at $p < 0.05$.

3. Results

3.1. Yeast and/or fluoxetine prevent LPS- and UCMS-induced immobility time in TST and FST paradigms

In both TST and FST paradigms, the immobility time is used as a measure of DLB in animal models (Omeiza et al., 2021, 2022; Ben-Azu et al., 2024). Interventions that decrease immobility time are often interpreted as potentially having antidepressant properties (Fig. 1).

In the TST paradigm (Fig. 1A–C), the administration of yeast and/or fluoxetine (5, 10, 20 mg/kg) significantly decreased immobility time [$F(5, 30) = 43.58$, $P < 0.0001$] in naive animals (Fig. 1 A). This demonstrates the ordinary effectiveness of these agents (when used separately or when combined) in reducing immobility in normal (non-depressed) animals. Of note, mice treated with both yeast (2%) and fluoxetine (20 mg/kg) showed the most significant reduction in immobility, suggesting a potential synergistic interaction between the two agents under this paradigm (Fig. 1 A). In the FST paradigm, however (Fig. 1 D), neither

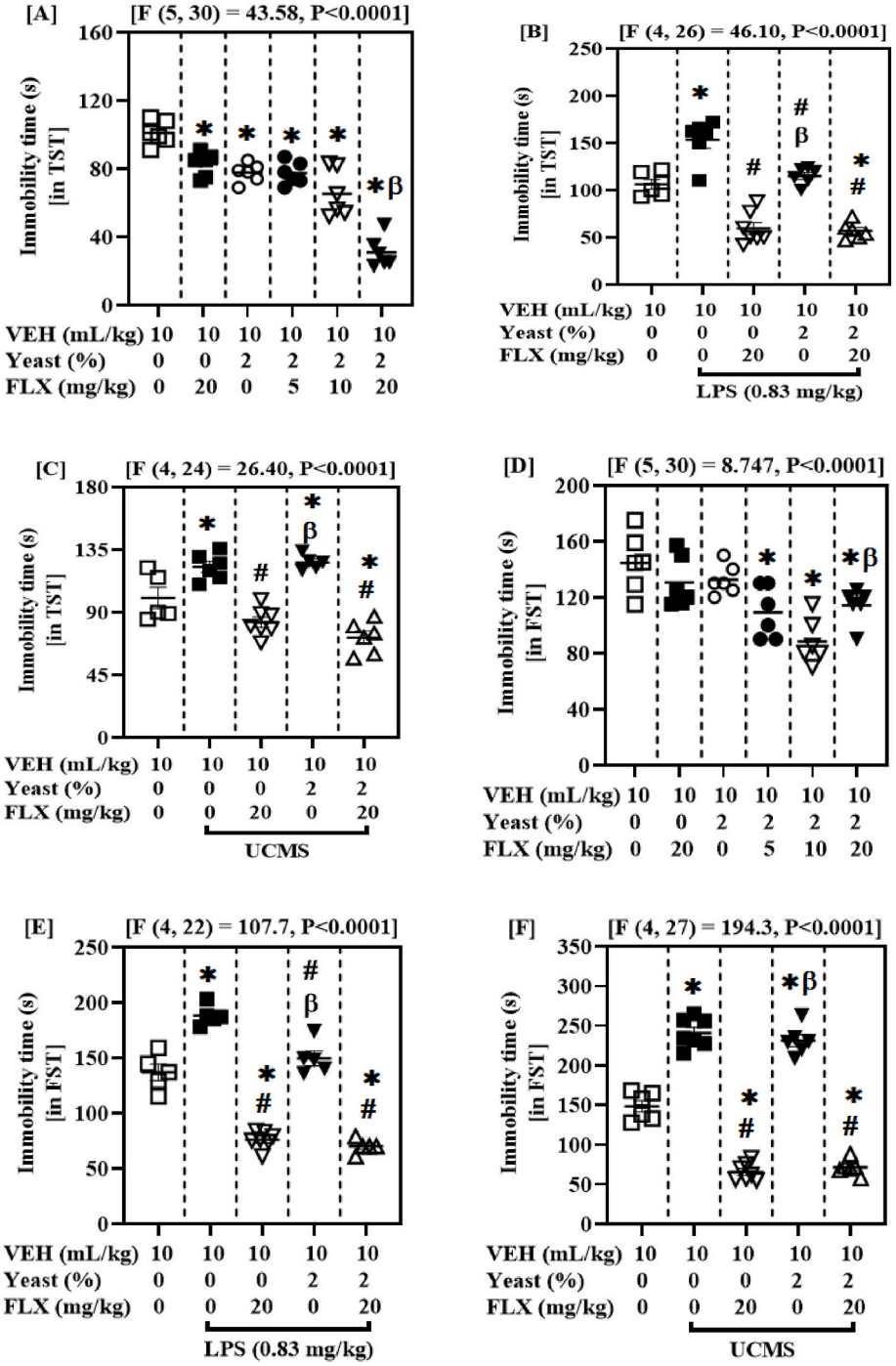


Fig. 1. Yeast and/or fluoxetine prevent LPS- and UCMS-induced immobility in TST and FST paradigms. Bars represent the mean ± S.E.M. $^{*}p < 0.0001$ compared to normal control group, $^{\#}p < 0.0001$ compared to negative control (LPS or UCMS) group and $^{\beta}p < 0.05$ compared to fluoxetine (20 mg/kg) group. VEH = vehicle; FLX = fluoxetine; TST = tail suspension test; FST = forced swimming test; LPS = lipopolysaccharide; UCMS = unpredictable chronic mild stress.

yeast nor fluoxetine (20 mg/kg) administered individually exhibited any discernible impact on immobility duration [F (5, 30) = 8.747, $P < 0.0001$] in naive animals. However, a significant reduction in immobility duration was observed when yeast and fluoxetine (all doses, though 20 mg/kg had more pronounced effect) were co-administered, also suggesting a potential synergistic effect between the two agents in non-depressed mouse under the FST paradigm (Fig. 1 D). Thus, we used only 20 mg/kg fluoxetine in stages II (LPS model) and III (UCMS model) of the DLB studies as it showed the most noticeable effect with yeast.

The immobility time in the TST and FST paradigms during LPS model of DLB are shown in Fig. 1 B and E. As illustrated in Fig. 1 B, LPS administration led to increased immobility time [F (4, 26) = 46.10, $P < 0.0001$] in negative control mice, indicative of a depressive-like state induced by LPS. However, the administration of yeast and/or fluoxetine effectively prevented this LPS-induced effect, with mice treated with fluoxetine and yeast showing further decreased immobility compared to normal control mice (Fig. 1 B), demonstrating the synergistic effect of these agents. Although yeast partly decreased immobility duration in LPS-exposed animals, its effectiveness was inferior to that of fluoxetine alone (Fig. 1 B). However, concurrent treatment with yeast and fluoxetine demonstrated complete prevention, showing even more effectiveness than fluoxetine alone in both LPS-exposed animal (Fig. 1 B). Besides, in the LPS-induced depression model of FST paradigm, animals in the negative control group demonstrated increased immobility duration [F (4, 22) = 107.7, $P < 0.0001$], whereas this trend was prevented in the groups pre-treated with yeast and/or fluoxetine, although the effect is more pronounced in the group that received a combination of yeast and fluoxetine (Fig. 1 E).

The immobility time in the TST and FST paradigms during UCMS model of DLB are shown in Fig. 1 C and F. Consistent with the findings from LPS exposure, mice exposed to UCMS also exhibited heightened immobility time in the TST paradigm [F (4, 24) = 26.40, $P < 0.0001$]. While yeast alone did not alleviate the adverse effects of UCMS exposure, fluoxetine alone as well as its combination with yeast demonstrated a preventive effect, leading to reduced immobility, with their combination producing more noticeable effect (Fig. 1 C), again demonstrating the synergistic effect of the two agents in UCMS model of DLB. In the FST paradigm, a notable increase in immobility duration [F (4, 27) = 194.3, $P < 0.0001$] was recorded in the negative control group, but while yeast alone failed to prevent this behavioral phenotype, its combination with fluoxetine markedly attenuated the adverse effects of UCMS (Fig. 1 F).

3.2. Yeast and/or fluoxetine prevent elevation of corticosterone in mice exposed to UCMS model of DLB

To confirm the establishment of UCMS in the animals, corticosterone, a hormone indicative of stress response in experimental animals (Nollet, 2021), was measured in the plasma. As depicted in Fig. 2, animals in the negative control group exhibited heightened concentration of corticosterone [F (4, 20) = 46.07, $P < 0.0001$]. However, this response was prevented in groups pre-treated with yeast and/or fluoxetine, with the combined treatment showing more pronounced effect than either of them. These data suggest that the interventions used in this study elicit an anti-stress-like effect, thereby enhancing the resilience of the animals and counteracting UCMS-induced DLB.

3.3. Yeast and/or fluoxetine prevent LPS- and UCMS-induced neuroinflammation in mice

As depicted in Fig. 3, the levels of TNF-α (Fig. 3A and B) and IL-6 (Fig. 3C and D) were elevated in the negative control animals of the LPS and UCMS models of DLB, respectively.

However, the elevation of TNF-α concentration [F (4, 10) = 58.61, $P < 0.0001$] was prevented in LPS-exposed animals treated with fluoxetine alone and its combination with yeast, but not in those that received

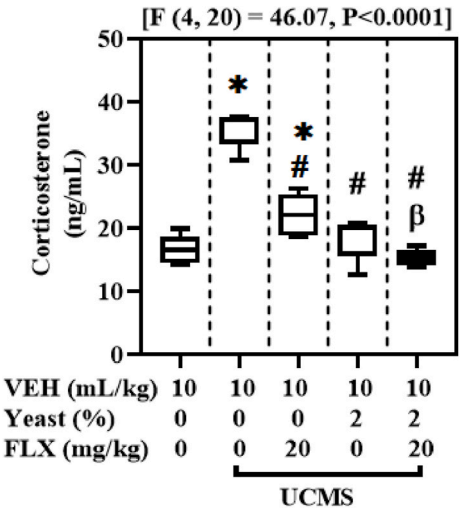


Fig. 2. Yeast and/or fluoxetine lower corticosterone concentration in mice exposed to UCMS model of DLB. Bars represent the mean ± S.E.M. $^{*}p < 0.0001$ compared to normal control group, $^{\#}p < 0.05$ compared to negative control group and $^{\beta}p < 0.0001$ compared to fluoxetine (20 mg/kg) group. VEH = vehicle; FLX = fluoxetine; UCMS = unpredictable chronic mild stress.

yeast alone (Fig. 3 A). Similarly, TNF-α concentration [F (4, 20) = 80.07, $P < 0.0001$] was decreased in UCMS-exposed animals treated with yeast and/or fluoxetine, with their combination producing the most noticeable effect that is comparable to the normal control in the UCMS model (Fig. 3 B).

Likewise, a reduction in IL-6 level was observed in both LPS- [F (4, 10) = 120.3, $P < 0.0001$] (Fig. 3 C) and UCMS-exposed [F (4, 20) = 384.8, $P < 0.0001$] (Fig. 3 D) animals treated with yeast and/or fluoxetine, with more pronounced effect noticeable in the group pre-treated with their combination that is comparable to the normal control group especially in the UCMS model, suggesting a synergistic effect.

3.4. Yeast and/or fluoxetine prevent LPS- and UCMS-induced oxidative stress in mice

The LPS and UCMS models have been implicated not only in the induction of neuroinflammation but also oxidative stress, contributing to various neurological conditions, including depression, in animals (Omeiza et al., 2021). In this study, we investigated the impact of yeast and/or fluoxetine on biomarkers of oxidative stress such as MDA, GSH, SOD, CAT, and NO in the brain tissues of mice (Fig. 4).

As shown in Fig. 4 A [F (4, 20) = 21.99, $P < 0.0001$] and **4 B** [F (4, 20) = 16.85, $P < 0.0001$], MDA concentrations in the brains of LPS- and UCMS-exposed mice were elevated compared to the normal control group. However, administration of yeast and/or fluoxetine, especially when combined, resulted in lower MDA concentrations, approaching levels observed in the normal control groups (Fig. 4A and B). This suggests that both yeast and fluoxetine, individually or in combination, have potential therapeutic effects in mopping reactive oxygen species (ROS), as evident by the reduction in MDA levels, which serves as a marker of lipid peroxidation, a process triggered by ROS (Omeiza et al., 2023).

Furthermore, these agents enhanced antioxidant capacity, as evident by the data presented in Fig. 4C–J. Mice exposed to LPS and UCMS experienced decreases in GSH levels (Fig. 4C and D), as well as in activities of CAT (Fig. 4E and F) and SOD (Fig. 4G and H). However, administration of yeast and/or fluoxetine prevented the detrimental effects induced by LPS [F (4, 20) = 16.67, $P < 0.0001$] (Fig. 4 C) and UCMS [F (4, 20) = 20.01, $P < 0.0001$] (Fig. 4 D) on GSH levels. Notably, yeast alone did not prevent LPS-induced reduction in GSH (Fig. 4 C). Similarly, while yeast alone did not prevent altered CAT activity [F (4,

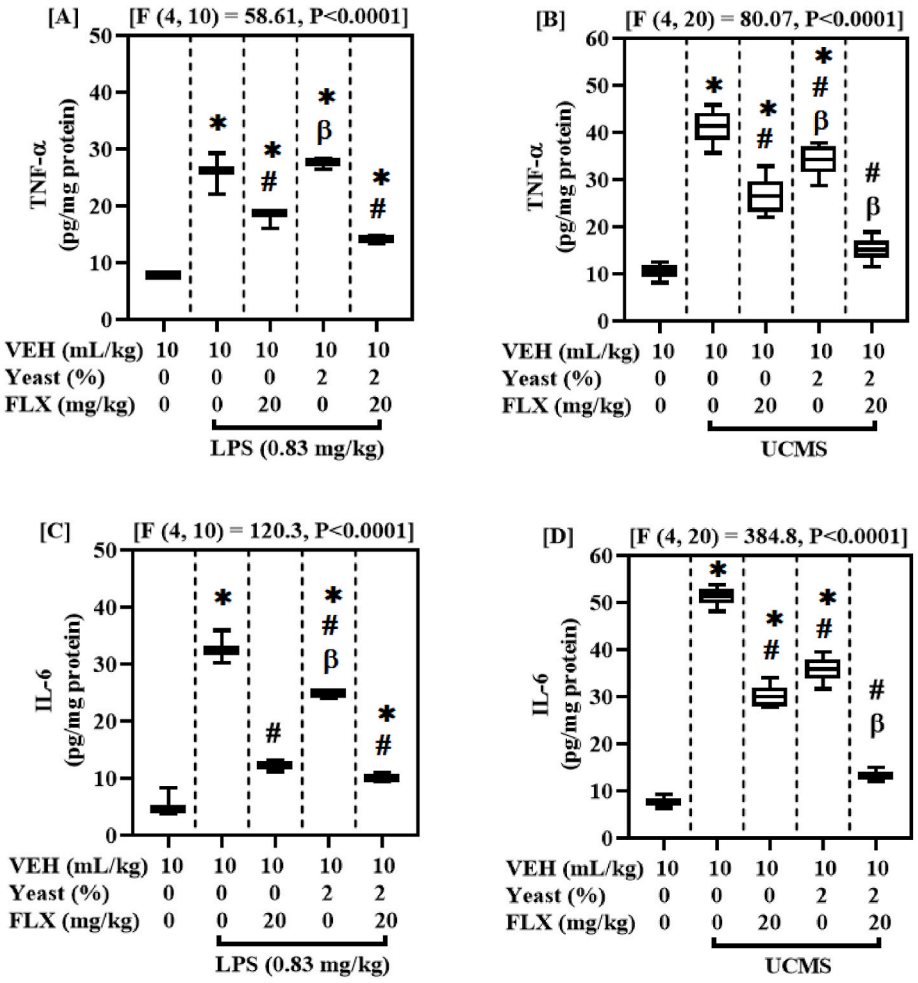


Fig. 3. Yeast and/or fluoxetine prevent LPS- and UCMS-induced neuro-inflammation in mice. Bars represent the mean ± S.E.M. $^{*}p < 0.0001$ compared to normal control group, $^{\#}p < 0.0001$ compared to negative control group and $^{\beta}p < 0.05$ compared to fluoxetine (20 mg/kg) group. VEH = vehicle; FLX = fluoxetine; TNF-α = Tumor necrosis factor-alpha; IL-6 = interleukin-6; LPS = lipopolysaccharide; UCMS = unpredictable chronic mild stress.

20) = 24.12, $P < 0.0001$] induced by LPS, pre-treatment with fluoxetine alone, as well as its combination with yeast, increased CAT activity in LPS-treatment mice (Fig. 4 E). Moreover, UCMS-exposed mice treated with yeast and/or fluoxetine also experienced an increase in CAT activity [F (4, 20) = 11.30, $P < 0.0001$] (Fig. 4 F).

Interestingly, SOD activity was also preserved in LPS- [F (4, 20) = 20.87, $P < 0.0001$] and UCMS- [F (4, 20) = 11.59, $P < 0.0001$] exposed mice treated with yeast and/or fluoxetine (Fig. 4G and H). Additionally, nitrite, which serves as an index of NO known to be implicated in both inflammation and oxidative stress, was modulated by yeast and/or fluoxetine in LPS- [F (4, 20) = 22.88, $P < 0.0001$] and UCMS- [F (4, 20) = 14.48, $P < 0.0001$] exposed mice (Fig. 4I–J).

Overall, our data suggest that co-administration of yeast and fluoxetine was the most effective among all treatments in mitigating the adverse effects of LPS and UCMS on oxidative stress biomarkers in mice (Fig. 4A–J).

3.5. Yeast and/or fluoxetine prevent LPS- and UCMS-induced AChE activity dysregulation in mice

Previously, the impacts of LPS- and UCMS-induced neuro-inflammation and oxidative stress in animal models of depression have been associated with neurotransmission disruption and cognitive impairment (Da Ré et al., 2020; Bakhtiari-Dovvombaygi et al., 2021). Therefore, in this study, we assessed the effects of yeast and/or fluoxetine on the brain AChE activity, serving as an index of acetylcholine cognitive function. As depicted in Fig. 5, animals in the negative control group exhibited heightened activity of AChE. However, this LPS- [F (4, 20) = 327.8, $P < 0.0001$] (Fig. 5 A) and UCMS- [F (4, 20) = 86.06, $P < 0.0001$] (Fig. 5 B) induced effects were prevented in groups treated with yeast and/or fluoxetine. Irrespective of these models of depression, the preventive decreases in AChE activity observed in all the treated animals were still not enough compared to the activity found in the normal control animals. Furthermore, co-treatment with yeast and fluoxetine exhibited a more pronounced anti-AChE activity compared to other preventive treatments, suggesting a synergistic effect on cholinergic function (Fig. 5A and B).

3.6. Yeast and/or fluoxetine prevent UCMS-induced CREB and MAPK activities dysregulation in mice

Dysregulation in the CREB and MAPK signaling pathways has emerged as prominent features in UCMS-induced depression models. These pathways play vital roles in regulating gene expression and cellular mechanisms associated with mood regulation and synaptic plasticity. In this study, we investigated the impact of yeast and/or fluoxetine on CREB and MAPK activities in the brain. As depicted in Fig. 6, animals exposed to UCMS without any intervention displayed a decrease in CREB activity [F (4, 20) = 12.67, $P < 0.0001$] (Fig. 6 A) and an increase in MAPK activity [F (4, 20) = 25.05, $P < 0.0001$] (Fig. 6 B). However, yeast and/or fluoxetine administration elevated CREB activity (Fig. 6 A), but not MAPK activity (except in mice that received their

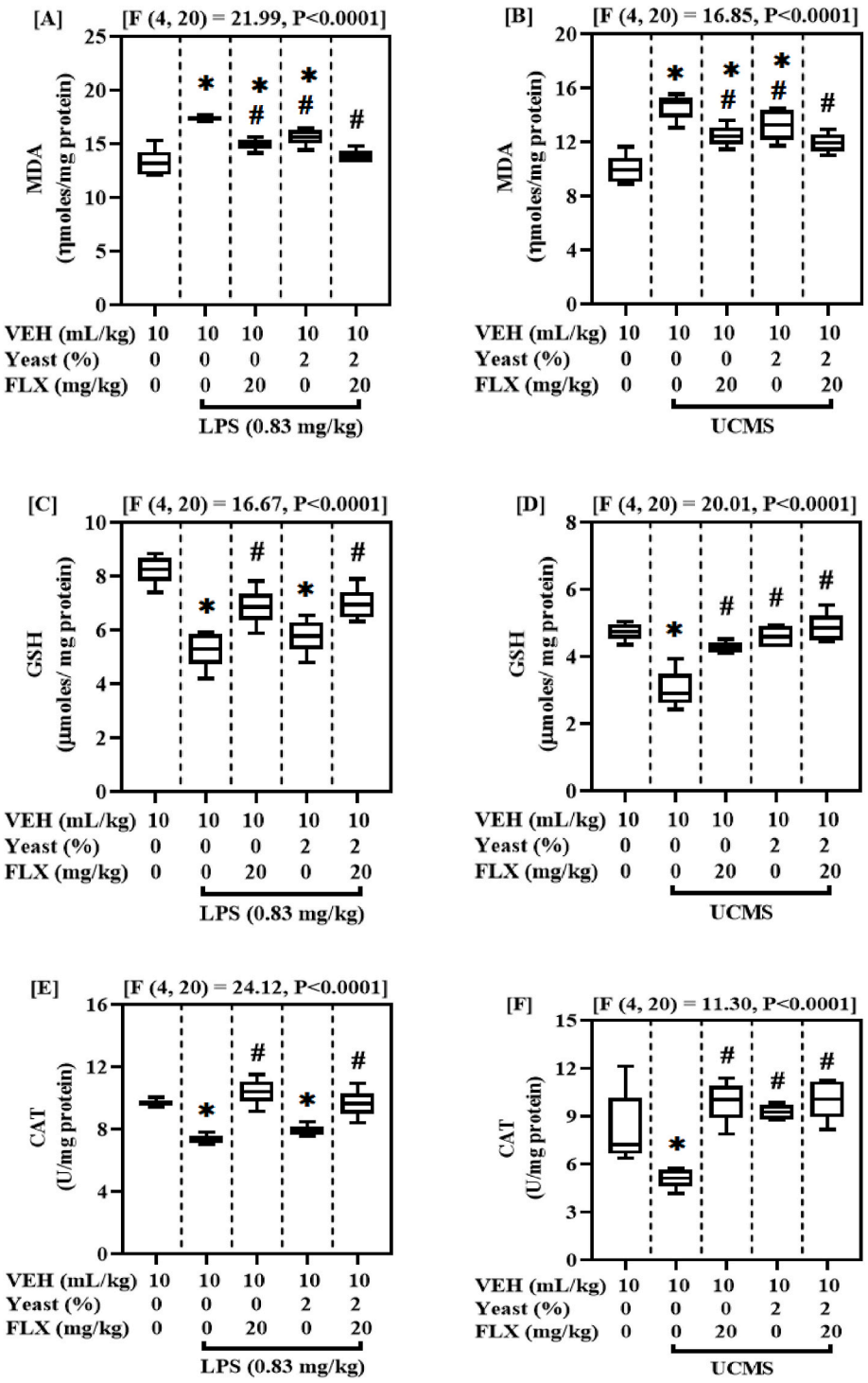


Fig. 4. Yeast and/or fluoxetine prevent LPS- and UCMS-induced oxidative stress in mice. Bars represent the mean ± S.E.M. $^{*}p < 0.0001$ compared to normal control group, $^{\#}p < 0.0001$ compared to negative control group and $^{\beta}p < 0.05$ compared to fluoxetine (20 mg/kg) group. VEH = vehicle; FLX = fluoxetine; MDA = Malondialdehyde; GSH = reduced glutathione; SOD = Superoxide dismutase; CAT = Catalase; NO = Nitric oxide; LPS = lipopolysaccharide; UCMS = unpredictable chronic mild stress.

combination to demonstrate synergistic effect) (Fig. 6B), in UCMS-treated mice.

4. Discussion

Behavioral tests like TST and FST are widely acknowledged as intuitive paradigms for evaluating antidepressant action of compounds, nutraceuticals and plant-based extracts in animal models of depression, including the UCMS and LPS models. A meta-analysis of various behavioral tests used to assess DLB in animals demonstrated that TST and FST are the most frequently utilized paradigms, accounting for 115 and 140 studies, respectively, out of the 502 studies meta-analyzed across 18 methods (Yin et al., 2023). Both tests share a similar theoretical basis, measuring the time spent stationary in an inescapable

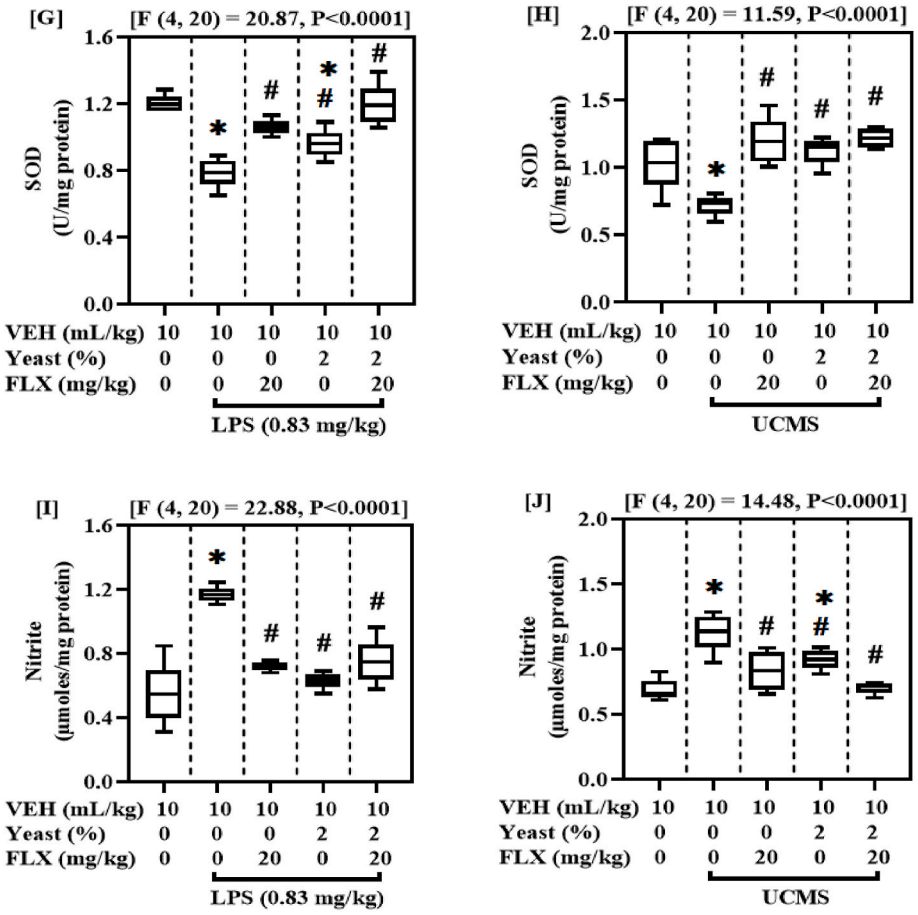


Fig. 4. (*continued*).

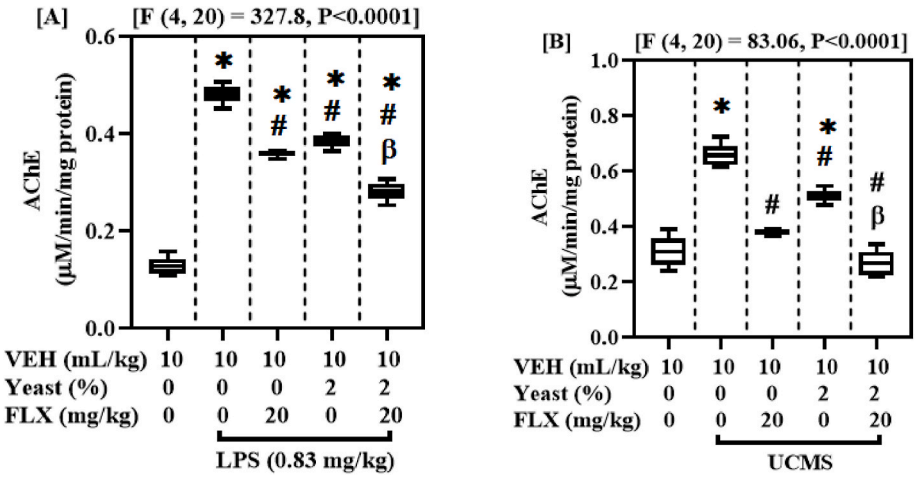


Fig. 5. Yeast and/or fluoxetine prevent LPS- and UCMS-induced AChE dysfunction in mice. Bars represent the mean ± S.E.M. $^{*}p < 0.0001$ compared to normal control group, $^{\#}p < 0.05$ compared to negative control group and $^{\beta}p < 0.0001$ compared to fluoxetine (20 mg/kg) group. VEH = vehicle; FLX = fluoxetine; AChE = Acetylcholinesterase; LPS = lipopolysaccharide; UCMS = unpredictable chronic mild stress.

environment, which reflects despair-like behaviors indicative of depressive psychomotor retardation (Unal and Canbeyli, 2019). While TST and FST are valuable for assessing behavioral despair, the underlying mechanisms of depression involve complex biochemical and molecular pathways. Studies have demonstrated the contribution of oxidative stress to depression (Maes et al., 2011), as it leads to neurotransmitter metabolism dysfunction in the brain, particularly eliciting an elevation in the AChE (Wang et al., 2019, Omeiza et al., 2021; Adebayo et al., 2023). The association of NO to inflammation has been underscored, as pro-inflammatory cytokines like TNF-α and IL-6 activate the inducible isoform of nitric oxide synthase (iNOS) (Isibor et al., 2022; Oredeko et al., 2023). The pro-inflammatory cytokines increase central monoamine metabolism and potently activate the hypothalamic-pituitary-adrenal (HPA) axis, showing the disturbance of both systems in depression. Moreover, CREB is known to be upregulated in the hippocampus by chronic anti-depressant treatment because

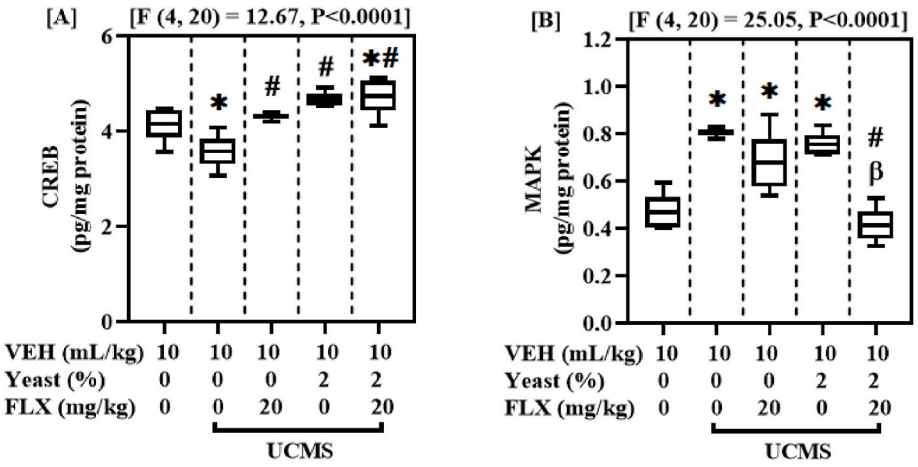


Fig. 6. Yeast and/or fluoxetine prevent UCMS-induced CREB and MAPK activities dysregulation in mice. Bars represent the mean ± S.E.M. $^{*}p < 0.0001$ compared to normal control group, $^{\#}p < 0.0001$ compared to negative control group and $^{\beta}p < 0.05$ compared to fluoxetine (20 mg/kg) group. VEH = vehicle; FLX = fluoxetine; CREB = cAMP response element-binding protein; MAPK = mitogen-activated protein kinase; UCMS = unpredictable chronic mild stress.

monoaminergic signaling pathways act via G-protein to alter adenylyl cyclase activity, which then stimulate the formation of cAMP. The cAMP activates protein kinase A and consequently, CREB that is constitutively expressed following antidepressant treatments (Carlezon et al., 2005; Tardito et al., 2006). Another pathway, the MAPK, has also been well-documented to be activated by UCMS model of DLB (Wu et al., 2018; Yang et al., 2019; Liaqat et al., 2022; Li et al., 2023).

In the present study, we assessed the immobility time in the TST and FST paradigms to understand whether yeast, when used alone or combined with fluoxetine as pre-treatment(s), could prevent DLB using LPS and UCMS experimental mice models. We also investigated the involvement of oxido-inflammatory pathways, CREB and MAPK pathways in the possible anti-depressant effect of yeast (either alone or in combination with fluoxetine). We noted that pre-treatment of mice with either yeast or fluoxetine or both prevents the induction of DLB by both LPS and UCMS. This observation shows that yeast and fluoxetine, either when used separately or when combined, could prevent DLB and the associated oxido-inflammatory processes. However, while yeast alone is not as potent as fluoxetine alone, their combination produced better effect than either of them. This suggests a synergistic effect of the two substances, prompting a recommendation of yeast-enriched diet in patients receiving fluoxetine treatment for better management of depression, subject to further confirmation by a clinical study.

While mice models have been reported to be more suitable than rats in the application of neuroinflammation-induced depression, different mice strains have also been used in preclinical studies of depression. It is established that the sensitivity of mouse strains to antidepressants, the behavioral, and the mechanisms of actions of drugs differ among animal strains (Jin et al., 2017). A meta-analysis of 168 studies showed that C57BL/6 mice were the most frequently used strain in the LPS-induced depression model (frequency 86, accounting for 51.19%), followed by ICR mice (frequency 40, accounting for 23.81%) and Swiss mice (frequency 22, accounting for 13.10%). Balb/c mice, which is the mice strain used in this study, is one of the less commonly-used strains by others, accounting for only 12 studies (7.14%) out of 168 studies meta-analyzed (Yin et al., 2023). Another study comparing the susceptibility of outbred and inbred mice to stress-induced DLB gave more credence to C57BL/6, having higher immobility values (Lucki et al., 2001). Also, the intraperitoneal injection of LPS, which has been shown to be better than other routes, induces peritoneal inflammation that leads to DLB within 24 h via activation of the brainstem, hypothalamus, and the limbic structures via the vagal afferents (Yin et al., 2023). *In vivo* studies have shown that LPS (when administered either centrally or peripherally) acts as a pathogen-associated molecular pattern (PAMP) that induces peripheral inflammation by activating monocytes, macrophages, endothelial cells, and epithelial cells, which then stimulate the cellular signaling systems to increase various cytokines and pro-inflammatory mediators (Cavaillon, 2018). These peripheral inflammatory signals have been demonstrated to reach the CNS via the endothelial cells or the blood-brain barrier to induce neuroinflammation (Tan et al., 2021) and consequently, depression (Mariani et al., 2022). It is also established that LPS elicits production of pro-inflammatory cytokines and DLB at 6 h of administration, which peak at 24 h (Shirayama et al. 2015).

In this study, we chose Balb/c strain of mice to further confirm the potency of LPS to elicit neuroinflammation-induced DLB in this less-commonly used strain so as to further our understanding about whether strain differences could negatively impact this novel animal model of depression. We also tried to know if Balb/c strain is suitable for stress-induced DLB model. Similar to studies that assessed the immobility time in FST of C57BL/6, ICR, and Swiss mice stains given single injection of different doses (0.5 mg/kg or 0.83 mg/kg) of LPS (Yin et al., 2023), we also noted that single injection of 0.83 mg/kg LPS significantly increased the immobility time in both the TST and FST tests within 24 h, establishing a successful induction of DLB within this period in Balb/c strain. We also noted increased immobility time in the TST and FST of Balb/c mice subjected to UCMS. These observations in both LPS and UCMS models of DLB support the contention that neuroinflammation- and stress-induced DLB elicited by LPS and UCMS respectively are relevant in many mice strains, including Balb/c strain. Our finding is consistent with previous studies that report no difference in the immobility of different mice strains (David et al., 2003). This finding is clinically relevant because the knowledge of the appropriate experimental animal strain is crucial to preclinical determination of anti-depressant activity of drugs and other plants of ethnopharmacological potentials.

It has been well-established with various DLB models that fluoxetine, a well-known antidepressant drug, elicits its effects via suppression of pro-inflammatory cytokines (Nabirumbi et al., 2024). This anti-depressant function of fluoxetine was confirmed in our present study. Consistent with previous studies, we noted that fluoxetine decreased the immobility time in normal (non-depressed) mice, and its pretreatment in mice prevented LPS- and UCMS-induced DLB during both TST and FST via attenuation of oxido-inflammation and CREB pathways. The ability of fluoxetine to modulate these pathways had already been associated with its elevation of serotonin, which then inhibit the production of pro-inflammatory cytokines (Kenis and Maes, 2002). While it has been said that the gut is known to produce about

95% of the body's serotonin, thus its tag as the second brain, our study is limited by the absence of data on the serotonin level in various groups. However, the attenuation of LPS- and UCMS-induced elevation of AChE is by fluoxetine is noticeable, demonstrating that it restored synaptic integrity.

Yeast, which is a eukaryotic probiotic that can adhere to the gastrointestinal system, has glucan as a component of its cell wall, which modulates the immune system to improve the immune functions (Stier et al., 2014). Its probiotic effect in some diseased conditions has recently attracted scientific attention due to their resistance to antibiotics, giving them advantage over bacteria-based probiotics. Microorganisms in the genera *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* belong to the "generally regarded as safe (GRAS) category and are cultivable components of human microbiota (Amara and Shibl, 2015). Particularly, *S. cerevisiae* (from which our study's yeast was made) has beneficial effects on inflammatory bowel diseases (Gayathri et al., 2020), entheropatogenic bacteria (Roussel et al., 2018), and pathogenic fungi (Abdel-Kareem et al., 2019) via inhibition of pathogen adherence to epithelial cells (Kunyeit et al., 2019), inhibition of pathogen growth (Abdel-Kareem et al., 2019), inhibition of filamentation and biofilm development (Kunyeit et al., 2019), immunomodulatory activity (Roselletti et al., 2019a,b), and reduction of toxin production (Abdel-Kareem et al., 2019). Many studies have demonstrated the use of *S. cerevisiae*-based yeast to treat infectious diseases like Candida vaginitis and colitis, and that it prevents inflammatory processes via inhibition of hyphal growth-associated genes (hyphal wall protein 1 and extent of cell elongation 1) and secretory aspartyl proteinases (SAPs) 1 and 6 (Pericolini et al., 2015; Hu et al., 2023a,b). Other studies have reported its anti-infectious (Roussel et al., 2018) and anti-oxidant (Fakruddin et al., 2017) activities.

In this study, we investigated whether the well-established probiotic, anti-inflammatory and anti-oxidant effects of yeast could prevent DLB induced by alteration of oxido-inflammatory, CREB and MAPK pathways in LPS and UCMS models. We observed that yeast prevented LPS-induced inflammation and oxidative stress in mice, evident from the reduction in the pro-inflammatory markers (NO, IL-6, and TNF- α) and suppression of oxidative stress (reduction in MDA but increase in GSH, CAT, and SOD). We also noted that yeast attenuated UCMS-induced elevation of corticosterone, but not MAPK, while it prevented UCMS-induced reduction in CREB. This is consistent with the previous report of Kim et al. (2024) where *S. cerevisiae* suppressed LPS-induced NO production in 264.7 cells, thereby attenuating inflammation. It also aligns with previous observation that yeast possesses antioxidant and anti-inflammatory properties, which are associated with its glucans (Siesto et al., 2022) and anti-oxidant enzyme (Aluwong et al., 2013) contents. Our current study is limited by lack of data on the quantity of glucans and other components in the yeast used, which would have enabled us to relate them to the effects of yeast seen in this study.

However, it is worthy of note that the anti-inflammatory and anti-oxidative effects of yeast are not as potent as those of fluoxetine. Thus, we investigated the possible synergistic or additive effect of yeast and fluoxetine on LPS- and UCMS-induced DLB and the associated modulation of the oxido-inflammatory, CREB and MAPK pathways. We noted that pre-treatment of mice with a combination of yeast and fluoxetine led to better prevention of LPS- and UCMS-induced DLB and its associated derangement in the pathways. In order words, while fluoxetine alone produced better preventive effect than yeast alone, none of these agents was as effective as when they were combined in mice. In some cases, the combination of these agents even improved the parameters beyond the level of the normal control mice.

Taken together, this study provides the first evidence that supplementation of fluoxetine treatment with yeast provides better antidepressant effect in LPS and UCMS models of DLB. It has a potential clinical relevance, if confirmed in human studies, that placement of depressed patients in yeast-enriched diet could produce better antidepressant effect during fluoxetine treatment. Despite the novelty and strength of this study, it is limited by absence of dose-response data of yeast on the parameters reported and we recommend this for further studies to enrich our understanding on the potential antidepressant effect of yeast.

Ethical approval

The experimental protocol adhered to the standard procedures established by the Principle National Institution of Health and received ethical approval with the number UI-ACUREC/086–0921/13 from the University of Ibadan Animal Care and Use Research Ethics Committee. Every effort was made to uphold the principles of the 3Rs in animal research, prioritizing the humane treatment of the experimental animals.

Author contributions

AP: Conceptualization, Experimental Design, Data curation, Investigation, Methodology and Writing – original draft, All authors read the reviewed manuscript and approved it for publication; NAO: Data Analysis, Graphical Abstract Creating, Writing – original draft Writing – review & editing, All authors read the reviewed manuscript and approved it for publication; AMA: Experimental Design, Biochemical Analysis, and Supervision, All authors read the reviewed manuscript and approved it for publication; PAA: Data curation, and Biochemical Analysis, All authors read the reviewed manuscript and approved it for publication; AIA: Data Analysis, Writing – original draft Writing – review & editing, and Revision of the Manuscript, All authors read the reviewed manuscript and approved it for publication; EOI: Conceptualization, Experimental Design, Supervision, and Writing – review & editing. All authors read the reviewed manuscript and approved it for publication.

Availability of data and materials

The authors declare that the databases that support the results will be available when requested from the corresponding author.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abdel-Kareem, M.M., Rasmey, A.M., Zohri, A.A., 2019. The action mechanism and biocontrol potentiality of novel isolates of Saccharomyces cerevisiae against the aflatoxigenic Aspergillus flavus. Lett. Appl. Microbiol. 68 (2), 104–111.

Abdel-Kareem, M.M., Rasmey, A.M., Zohri, A.A., 2019. The action mechanism and biocontrol potentiality of novel isolates of Saccharomyces cerevisiae against the aflatoxigenic Aspergillus flavus. Lett. Appl. Microbiol. 68, 104–111. https://doi.org/10.1111/lam.13105.

Adebayo, O.G., Otene, C.I., Emmanuel, M.U., Onwuka, F.C., Aduema, W., Ben-Azu, B., Omeiza, N.A., Onasanwo, S.A., 2023. Chronic intermittent oxygen deprivation alters hippocampal cholinergic and glutamatergic system via oxido-inflammatory burden and HIF-1a/Bcl-2 activity in hypothyroid mice: ameliorative role of Ginkgo biloba supplement. Phytomedicine 3 (2), 100451.

Alemayehu, F., Ambaw, F., Gutema, H., 2019. Depression and associated factors among prisoners in Bahir Dar Prison, Ethiopia. BMC Psychiatr. 19, 1–7.

Aluwong, T., Kawu, M., Raji, M., Dzenda, T., Govwang, F., Sinkalu, V., Ayo, J., 2013. Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. Antioxidants 2 (4), 326–339.
Amara, A.A., Shibl, A., 2015. Role of Probiotics in health improvement, infection control and disease treatment and management. Saudi Pharmaceut. J. 23 (2), 107–114.
Bakhtiari-Dovvombaygi, H., Izadi, S., Zare, M., Asgari Hassanlouei, E., Dinpanah, H., Ahmadi-Soleimani, S.M., Beheshti, F., 2021. Vitamin D3 administration prevents memory deficit and alteration of biochemical parameters induced by unpredictable chronic mild stress in rats. Sci. Rep. 11 (1), 16271.
Baune, B.T., Stuart, M., Gilmour, A., Wersching, H., Heindel, W., Arolt, V., Berger, K., 2012. The relationship between subtypes of depression and cardiovascular disease: a systematic review of biological models. Transl. Psychiatry 2 (3), e92 e92.
Ben-Azu, B., Adebesin, A., Moke, G.E., Ojiokor, V.O., Olusegun, A., Jarikre, T.A., Akinluyi, E.T., Olukemi, O.A., Omeiza, N.A., Nkenchor, P., Niemogha, A.R., 2024. Alcohol exacerbates psychosocial stress-induced neuropsychiatric symptoms: attenuation by geraniol. Neurochem. Int. 105748.
Branyan, K.W., DeVallance, E.R., Lemaster, K.A., Skinner, R.C., Bryner, R., Olfert, I.M., et al., 2018. Role of chronic stress and exercise on microvascular function in metabolic syndrome. Med. Sci. Sports Exerc. 50 (5), 957.
Carlezon, W.A., Duman, R.S., Nestler, E.J., 2005. The many faces of CREB. Trends Neurosci. 28 (8), 436–445.
Cavaillon, J.M., 2018. Exotoxins and endotoxins: inducers of inflammatory cytokines. Toxicon 149, 45–53.
Crane, C., Barnhofer, T., Duggan, D.S., Eames, C., Hepburn, S., Shah, D., Williams, J.M. G., 2014. Comfort from suicidal cognition in recurrently depressed patients. J. Affect. Disord. 155, 241–246.
Da Ré, C., Souza, J.M., Fróes, F., Taday, J., Dos Santos, J.P., Rodrigues, L., et al., 2020. Neuroinflammation induced by lipopolysaccharide leads to memory impairment and alterations in hippocampal leptin signaling. Behav. Brain Res. 379, 112360.
David, D.J.P., Renard, C.E., Jolliet, P., Hascoët, M., Bourin, M., 2003. Antidepressant-like effects in various mice strains in the forced swimming test. Psychopharmacology 166, 373–382.
De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A.J., Green, W., et al., 2015. Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nat. Commun. 6 (1), 7735.
Emslie, G.J., Heiligenstein, J.H., Wagner, K.D., Hoog, S.L., Ernest, D.E., Brown, E., et al., 2002. Fluoxetine for acute treatment of depression in children and adolescents: a placebo-controlled, randomized clinical trial. J. Am. Acad. Child Adolesc. Psychiatr. 41 (10), 1205–1215.
Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., et al., 2015. Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18 (7), 965–977.
Fakruddin, M.D., Hossain, M.N., Ahmed, M.M., 2017. Antimicrobial and antioxidant activities of Saccharomyces cerevisiae IFST062013, a potential probiotic. BMC Compl. Alternative Med. 17, 1–11.
Gayathri, R., Aruna, T., Malar, S., Shilpa, B., Dhanasekar, K.R., 2020. Efficacy of Saccharomyces cerevisiae CNCM I-3856 as an add-on therapy for irritable bowel syndrome. Int. J. Colorectal Dis. 35, 139–145.
Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 126 (1), 131–138.
Grigoleit, J.S., Kullmann, J.S., Wolf, O.T., Hammes, F., Wegner, A., Jablonowski, S., et al., 2011. Dose-dependent effects of endotoxin on neurobehavioral functions in humans. PLoS One 6 (12), e28330.
Hu, T., Wang, S., Bing, J., Zheng, Q., Du, H., Li, C., et al., 2023a. Hotspot mutations and genomic expansion of ERG11 are major mechanisms of azole resistance in environmental and human commensal isolates of Candida tropicalis. Int. J. Antimicrob. Agents 62 (6), 107010.
Hu, Q., Yu, L., Zhai, Q., Zhao, J., Tian, F., 2023b. Anti-inflammatory, barrier maintenance, and gut microbiome modulation effects of Saccharomyces cerevisiae QHNLD8L1 on DSS-induced ulcerative colitis in mice. Int. J. Mol. Sci. 24 (7), 6721.
Isibor, H., Ajayi, A.M., Ben-Azu, B., Omeiza, N.A., Ademola, A.P., Umukoro, S., 2022. D-ribose-L-cysteine reduces oxidative stress and inflammatory cytokines to mitigate liver damage, and memory decline induced by copper sulfate in mice. J. Trace Elem. Med. Biol. 73, 127001.
Isingrini, E., Belzung, C., Freslon, J.L., Machet, M.C., Camus, V., 2012. Fluoxetine effect on aortic nitric oxide-dependent vasorelaxation in the unpredictable chronic mild stress model of depression in mice. Psychosom. Med. 74 (1), 63–72.
Jayatissa, M.N., Henningsen, K., Nikolajsen, G., West, M.J., Wiborg, O., 2010. A reduced number of hippocampal granule cells does not associate with an anhedonia-like phenotype in a rat chronic mild stress model of depression. Stress 13 (2), 95–105.
Jin, Z.L., Chen, X.F., Ran, Y.H., Li, X.R., Xiong, J., Zheng, Y.Y., et al., 2017. Mouse strain differences in SSRI sensitivity correlate with serotonin transporter binding and function. Sci. Rep. 7 (1), 8631.
Johansen, P.G., Owusu-Kwarteng, J., Parkouda, C., Padonou, S.W., Jespersen, L., 2019. Occurrence and importance of yeasts in indigenous fermented food and beverages produced in sub-Saharan Africa. Front. Microbiol. 10, 469794.
Kenis, G., Maes, M., 2002. Effects of antidepressants on the production of cytokines. Int. J. Neuropsychopharmacol. 5 (4), 401–412.
Kim, M., An, J., Shin, S.A., Moon, S.Y., Kim, M., Choi, S., et al., 2024. Anti-inflammatory effects of TP1 in LPS-induced Raw264.7 macrophages. Appl. Biol. Chem. 67, 16. https://doi.org/10.1186/s13765-024-00873-y.
Kryst, J., Majcher-Maślanka, I., Chocyk, A., 2022. Effects of chronic fluoxetine treatment on anxiety-and depressive-like behaviors in adolescent rodents–systematic review and meta-analysis. Pharmacol. Rep. 74 (5), 920–946.
Kunyeit, L., Kurrey, N.K., Anu-Appaiah, K.A., Rao, R.P., 2019. Probiotic yeasts inhibit virulence of non-albicans Candida species. mBio 10 (5), 10–1128.
Lach, G., Schellekens, H., Dinan, T.G., Cryan, J.F., 2018. Anxiety, depression, and the microbiome: a role for gut peptides. Neurotherapeutics 15 (1), 36–59.
Li, X., Teng, T., Yan, W., Fan, L., Liu, X., Clarke, G., et al., 2023. AKT and MAPK signaling pathways in hippocampus reveals the pathogenesis of depression in four stress-induced models. Transl. Psychiatry 13 (1), 200.
Liang, S., Wang, T., Hu, X., Li, W., Jin, F., Wang, L., 2012. Microorganism and behavior and psychiatric disorders. Adv. Psychol. Sci. 20 (1), 75.
Liang, S., Wu, X., Hu, X., Wang, T., Jin, F., 2018. Recognizing depression from the microbiota–gut–brain axis. Int. J. Mol. Sci. 19 (6), 1592.
Liaqat, H., Parveen, A., Kim, S.Y., 2022. Antidepressive effect of natural products and their derivatives targeting BDNF-TrkB in gut–brain axis. Int. J. Mol. Sci. 23 (23), 14968.
Lucki, I., Dalvi, A., Mayorga, A.J., 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology 155, 315–322.
Maes, M., Galecki, P., Chang, Y.S., Berk, M., 2011. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 35 (3), 676–692.
Mao, M., Li, S., Zong, M., Qiu, L., Yang, J., Xia, J., et al., 2019. Two-hit model of postintensive care syndrome induced by lipopolysaccharide challenge and subsequent chronic unpredictable stress in mice. Int. Immunopharm. 70, 446–458.
Mariani, N., Everson, J., Pariante, C.M., Borsini, A., 2022. Modulation of microglial activation by antidepressants. J. Psychopharmacol. 36 (2), 131–150.
Mazza, M.G., Palladini, M., De Lorenzo, R., Magnaghi, C., Poletti, S., Furlan, R., et al., 2021. Persistent psychopathology and neurocognitive impairment in COVID-19 survivors: effect of inflammatory biomarkers at three-month follow-up. Brain Behav. Immun. 94, 138–147.
Mineur, Y.S., Belzung, C., Crusio, W.E., 2006. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav. Brain Res. 175 (1), 43–50.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (10), 3170–3175.
Moron, M.S., Depierre, J.W., Mannervik, B., 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et biophysica acta (BBA)-general subjects 582 (1), 67–78.
Motlhanka, K., Zhou, N., Lebani, K., 2018. Microbial and chemical diversity of traditional non-cereal based alcoholic beverages of Sub-Saharan Africa. Beverages 4 (2), 36.
Mutlu, O., Gumuslu, E., Ulak, G., Celikyurt, I.K., Kokturk, S., Kır, H.M., et al., 2012. Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. Life Sci. 91 (25–26), 1252–1262.
Nabirumbi, R., Onohuean, H., Drago, K.C., Alagbonsi, A.I., Adedeji, A.A., 2024. Fluoxetine attenuates stress-induced depression-like behavior due to decrease in pro-inflammatory cytokines in male rats. Sci. Prog. 107 (1), 00368504241234786.
Nollet, M., 2021. Models of depression: unpredictable chronic mild stress in mice. Current protocols 1 (8), e208.
O'Leary, O.F., Cryan, J.F., 2013. Towards translational rodent models of depression. Cell Tissue Res. 354, 141–153.
Okhawa, H., Ohishi, N., Yagi, K., 1979. Reaction of linoleic acid hydroperoxides with thiobarbituric acids. Anal. Biochem. 95 (2), 351–354.
Omeiza, N.A., Abdulrahim, H.A., Alagbonsi, A.I., Ezurike, P.U., Soluoku, T.K., Isiabor, H., Alli-oluwafuyi, A.A., 2021. Melatonin salvages lead-induced neuro-cognitive shutdown, anxiety, and depressive-like symptoms via oxido-inflammatory and cholinergic mechanisms. Brain and Behavior 11 (8), e2227.
Omeiza, N.A., Bakre, A.G., Abdulrahim, H.A., Isibor, H., Ezurike, P.U., Sowunmi, A.A., et al., 2022. Pretreatment with Carpolobia lutea ethanol extract prevents schizophrenia-like behavior in mice models of psychosis. J. Ethnopharmacol. 295, 115432.
Omeiza, N.A., Bakre, A., Ben-Azu, B., Sowunmi, A.A., Abdulrahim, H.A., Chimezie, J., et al., 2023. Mechanisms underpinning Carpolobia lutea G. Don ethanol extract's neurorestorative and antipsychotic-like activities in an NMDA receptor antagonist model of schizophrenia. J. Ethnopharmacol. 301, 115767.
Oredeko, O.M., Omeiza, N.A., Ben-Azu, B., Ogiogio, H.A., Falade, J., Aderibigbe, A.O., Gbotosho, G.O., 2023. Anthocleista vogelii (Planch) stem bark ethanol extract prevents inflammatory and pain phenotypes in experimental rodent models by modulating oxido-inflammatory mediators. Phytomedicine 3 (3), 100475.
Painsipp, E., Köfer, M.J., Sinner, F., Holzer, P., 2011. Prolonged depression-like behavior caused by immune challenge: influence of mouse strain and social environment. PLoS One 6 (6), e20719.
Peng, G.J., Tian, J.S., Gao, X.X., Zhou, Y.Z., Qin, X.M., 2015. Research on the pathological mechanism and drug treatment mechanism of depression. Curr. Neuropharmacol. 13 (4), 514–523.
Pericolini, E., Gabrielli, E., Roselletti, E., Luciano, E., Sabbatini, S., Moser, C., et al., 2015. A role for secretory aspartyl proteinase of Candida albicans in mouse vaginal inflammation and vaginal candidiasis. In: Human Fungal Pathogens Molecular Mechanisms of Host-Pathogen Interactions and Virulence. FEBS.
Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47 (4), 379–391.
Rieder, R., Wisniewski, P.J., Alderman, B.L., Campbell, S.C., 2017. Microbes and mental health: a review. Brain Behav. Immun. 66, 9–17.
Roselletti, E., Monari, C., Sabbatini, S., Perito, S., Vecchiarelli, A., Sobel, J.D., Cassone, A., 2019a. A role for yeast/pseudohyphal cells of Candida albicans in the correlated expression of NLRP3 inflammasome inducers in women with acute vulvovaginal candidiasis. Front. Microbiol. 10, 489929.

Roselletti, E., Sabbatini, S., Ballet, N., Perito, S., Pericolini, E., Blasi, E., et al., 2019b. Saccharomyces cerevisiae CNCM I-3856 as a new therapeutic agent against oropharyngeal candidiasis. Front. Microbiol. 10, 1469.

Roussel, C., Sivignon, A., De Vallee, A., Garrait, G., Denis, S., Tsilia, V., et al., 2018. Anti-infectious properties of the probiotic Saccharomyces cerevisiae CNCM I-3856 on enterotoxigenic E. coli (ETEC) strain H10407. Appl. Microbiol. Biotechnol. 102, 6175–6189.

Shirayama, Y., Yang, C., Zhang, J.C., Ren, Q., Yao, W., Hashimoto, K., 2015. Alterations in brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in the brain regions of a learned helplessness rat model and the antidepressant effects of a TrkB agonist and antagonist. Eur. Neuropsychopharmacol 25 (12), 2449–2458.

Siesto, G., Pietrafesa, R., Infantino, V., Thanh, C., Pappalardo, I., Romano, P., Capece, A., 2022. In vitro study of probiotic, antioxidant and anti-inflammatory activities among indigenous Saccharomyces cerevisiae strains. Foods 11 (9), 1342.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem. 47 (2), 389–394. https://doi.org/10.1016/0003-2697(72)90132-7.

Slavich, G.M., Irwin, M.R., 2014. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. Psychol. Bull. 140 (3), 774.

Slyepchenko, A., Maes, M., Jacka, F.N., Köhler, C.A., Barichello, T., McIntyre, R.S., et al., 2017. Gut microbiota, bacterial translocation, and interactions with diet: pathophysiological links between major depressive disorder and non-communicable medical comorbidities. Psychother. Psychosom. 86 (1), 31–46.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Stier, H., Ebbeskotte, V., Gruenwald, J., 2014. Immune-modulatory effects of dietary yeast beta-1, 3/1, 6-D-glucan. Nutr. J. 13 (1), 38.

Sulakhiya, K., Kumar, P., Gurjar, S.S., Barua, C.C., Hazarika, N.K., 2015. Beneficial effect of honokiol on lipopolysaccharide induced anxiety-like behavior and liver damage in mice. Pharmacol. Biochem. Behav. 132, 79–87. https://doi.org/10.1016/j.pbb.2015.02.015.

Takada, A., Shimizu, F., Masuda, J., 2018. Measurement of plasma tryptophan metabolites: clinical and experimental application for depression and stress states assessment. Melatonin—Molecular Biology, Clinical and Pharmaceutical Approaches. IntechOpen, London, pp. 143–160.

Tan, X.X., Qiu, L.L., Sun, J., 2021. Research progress on the role of inflammatory mechanisms in the development of postoperative cognitive dysfunction. BioMed Res. Int. 2021.

Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G., Popoli, M., 2006. Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. Pharmacol. Rev. 58 (1), 115–134.

Tian, T., Mao, Q., Xie, J., Wang, Y., Shao, W.H., Zhong, Q., Chen, J.J., 2022. Multi-omics data reveals the disturbance of glycerophospholipid metabolism caused by disordered gut microbiota in depressed mice. J. Adv. Res. 39, 135–145.

Unal, G., Canbeyli, R., 2019. Psychomotor retardation in depression: a critical measure of the forced swim test. Behav. Brain Res. 372, 112047.

Wang, X., Li, P., Ding, Q., Wu, C., Zhang, W., Tang, B., 2019. Observation of acetylcholinesterase in stress-induced depression phenotypes by two-photon fluorescence imaging in the mouse brain. J. Am. Chem. Soc. 141 (5), 2061–2068.

Woodward, A.T., Taylor, R.J., Abelson, J.M., Matusko, N., 2013. Major depressive disorder among older African Americans, Caribbean Blacks, and non-Hispanic Whites: secondary analysis of the national survey of American life. Depress. Anxiety 30 (6), 589–597.

Wu, Z., You, Z., Chen, P., Chen, C., Chen, F., Shen, J., Xu, H., 2018. Matrine exerts antidepressant-like effects on mice: role of the hippocampal PI3K/Akt/mTOR signaling. Int. J. Neuropsychopharmacol. 21 (8), 764–776.

Yalcin, I., Aksu, F., Belzung, C., 2005. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. Eur. J. Pharmacol. 514 (2–3), 165–174. https://doi.org/10.1016/j.ejphar.2005.03.029.

Yang, S.J., Song, Z.J., Wang, X.C., Zhang, Z.R., Wu, S.B., Zhu, G.Q., 2019. Curculigoside facilitates fear extinction and prevents depression-like behaviors in a mouse learned helplessness model through increasing hippocampal BDNF. Acta Pharmacol. Sin. 40 (10), 1269–1278.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., et al., 2012. Human gut microbiome viewed across age and geography. Nature 486 (7402), 222–227.

Yin, R., Zhang, K., Li, Y., Tang, Z., Zheng, R., Ma, Y., et al., 2023. Lipopolysaccharide-induced depression-like model in mice: meta-analysis and systematic evaluation. Front. Immunol. 14, 1181973.

Zalar, B., Blatnik, A., Maver, A., Klemenc-Ketiš, Z., Peterlin, B., 2018. Family history as an important factor for stratifying participants in genetic studies of major depression. Balkan J. Med. Genet. 21 (1), 5–12.